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NRL Report No. P-2364

NAVY DEPARTMENT

## Report on

A Controlled Laboratory Experiment <sup>Entitled</sup>  
to Compare Lesions Resulting from  
Application of  
Mustard, Lewisite, and Nitrogen Mustards  
to the Skin of the Forearms of Humans.

NAVAL RESEARCH LABORATORY  
ANACOSTIA STATION  
WASHINGTON, D. C.

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## ABSTRACT

Vapor cups containing mustard, Lewisite, and nitrogen mustard, ( $\text{HN}_3$ ), were employed to produce lesions of comparable size and severity on the fore-arms of humans. In two (2) other series of tests, drops of equal weight and drops of equal volume of each of the above vesicants were applied to the fore-arms of men to compare the vesicant powers of the agents and to study the type of lesion produced in each case. Color photographs, both still and motion picture, were taken throughout the experiment to show each lesion developing, reaching a peak and subsiding. To the best of our knowledge, no previous experiments have been performed by placing the three (3) basic types of gases (mustard, arsenicals, and nitrogen mustards) on the same individual, with systematic observation, including pictures, from the time of application of the gas through the development and healing of the lesion. It is felt that these observations will be an aid to other investigators who are either new in this field, or do not have the facilities for running this type of experiment.

AUTHORIZATION

1. This problem was authorized by Bureau of Ships letter, reference (a), and Bureau of Ships Project, reference (b).

Reference (a) BuShips S-S77-2(dz) Serial 811,  
of 17 December 1940.

Reference (b) BuShips Project No. 397/44  
of 2 February 1944.

OBJECT OF TEST

2. The object of this test was to produce and study the different types of lesions caused by the application of varying amounts of the three (3) basic types of vesicant gases; i.e., mustards, arsenicals, and nitrogen mustards, to the skin of the forearm of humans.

### DESCRIPTION OF MATERIAL UNDER TEST .

3-1. The equipment used was the standard Edgewood-type vapor cups with (2) drops of liquid vesicant on the blotter paper at the base of the cup, and the drods developed by the Rockefeller Institute for Medical Research using a platinum needle, the end of which had been cut off at right angles to the shaft. The drods were calibrated so that it was possible to deliver one (1) milligram and five-tenths (0.5) microliter amounts of each gas for the weight and volume experiments respectively.

3-2. For the photographs, two (2) standard Speed Graphic cameras with F 3.5 and F 4.5 lenses and a 16 mm Ciné Kodak motion picture camera with an F 2.7 lens were employed. The film used was 4" by 5" Kodachrome for the stills and 16 mm Kodachrome for the motion pictures. Two (2) Photoflood #4 lamps furnished the illumination. A permanent background and base for the arms of the men were set up so that angles and lighting of the different pictures would be as near the same as possible. The subject's number and the time after application of the gas were marked on the forearm for each picture. For the still pictures, it was necessary to take three (3) exposures for each picture required in order to insure having the proper color effect. When the films were sorted and edited, the best of each set of three (3) were retained for the report. The motion picture film was cut and re-spliced to produce continuous pictures of each lesion developing and subsiding.

### METHOD OF TEST

4. Because of the diversion of opinion of many investigators as to the type of lesion resulting from gases if applied by different methods, it was decided that the following procedure would be used:

- (1) Gas applied by means of so-called vapor cup
- (2) Gas applied in liquid form by equal weight  
(1 milligram)
- (3) Gas applied in liquid form by equal volume  
(0.5 microliters)

#### 4-1. Preliminary:

It was necessary to run preliminary screening tests to determine the proper times of application of

## METHOD OF TEST Continued)

the cups and the amount by weight and volume of the liquid vesicant needed to produce a lesion of the desired size and severity:

### (a) Vapor Cup Screening:

Cups, one (1) of H, two (2) of L, and one (1) of  $\text{HN}_3$ , set up as previously described, were applied to the forearms of one (1) man. Arbitrary times of ten (10) minutes for H, two (2) and six (6) minutes for L, and eighty (80) minutes for the  $\text{HN}_3$  were chosen. After twenty-four (24) hours, the H showed a vesicle (V)  $17 \times 18$  mm, the two-minute L numerous pinpoint vesicles (NPV)  $15 \times 16$  mm, the six-minute L a vesicle (V)  $22 \times 20$  mm, the  $\text{HN}_3$  a vesicle (V)  $15 \times 13$  mm. From these results, it was decided that for the actual experiment the cups should be applied ten (10) minutes for H, six (6) minutes for L, and one hundred (100) minutes for  $\text{HN}_3$ .

### (b) Equal Weight Screening:

Two (2) men were used in order to find the proper amount of liquid gas to apply to produce a lesion of the required size. Comparing applications of five-tenths (0.5), one (1), two (2), and three (3) milligrams of mustard to the forearms of the two (2) men, it was found that one (1) milligram was the amount desired. The next step was to apply one (1) milligram of each agent to the forearm of one (1) man to see if the lesions from all vesicants would be satisfactory, and to obtain enough information to set up a tentative schedule for intervals between observations and photographs.

### (c) Equal Volume Screening:

After the weight of one (1) milligram had been decided upon, a unit of volume of mustard comparable to one (1) milligram was chosen, this unit being five-tenths (0.5) microliters. A final screening test comparable to the one for equal weight was run by applying five-tenths (0.5) microliters of each vesicant to the forearm of one (1) man.

## 4-2. General:

From the results of the screening tests, it was decided to use twelve (12) men: six (6) for the vapor cup test, three (3) for the equal weight test, and three (3) for the equal volume test. The men had an application of each agent on the flexor surface of the left

## METHOD OF TEST (Continued)

forearm so that one (1) was at the mid-point, and the other equidistant from the mid-point and cubital fossa or wrist respectively. The position of application of each gas on the arm was rotated so that sensitivity of the different parts of the forearm could be determined and thus ruled out.

The lesions of the individuals were carefully guarded by keeping the men at leisure and using protective dressings on large vesicles. There was no unnecessary exposure to sunlight or active washing of the exposed area; swimming was prohibited. These steps were taken because it was desired to show a lesion developing, reaching a peak, and subsiding without interference by trauma, infection, etc.

The points particularly stressed in the early readings were as follows:

- (1) First visible change of the contaminated area -- erythema, blanching, etc.
- (2) Subjective symptoms -- itching, burning, etc.

During the entire experiment as many pictures as were deemed necessary by the medical officer were taken to provide a continuous series showing the most minute changes in the lesion.

Temperature and relative humidity were recorded throughout.

### 4-3. Experiment #1 - Vapor Cups.

As previously determined from screening tests, it was found that the order of severity of the vapor burns produced per unit time of application by the gases was as follows: Lewisite, mustard, nitrogen mustards. By calculation, it was determined that leaving the Lewisite cup on six (6) minutes, the mustard cup on ten (10) minutes, and the nitrogen mustard cup on one hundred (100) minutes would give approximately equally severe burns. Because it was the object of this experiment to produce burns in which any basic difference in characteristics could be noted and not difference in severity, the above

## METHOD OF TEST (Continued)

time limits for application of cups were used. It was necessary to use as zero (0) time in the experiment that time when the cups were removed. Therefore, the nitrogen mustard cup was applied first, in ninety (90) minutes the mustard cup, in ninety-four (94) minutes the Lewisite cup, and all were removed from the arm at the same time. Readings were taken every five (5) minutes up to two and one-fourth ( $2\frac{1}{4}$ ) hours, every fifteen (15) minutes to four and one-fourth ( $4\frac{1}{2}$ ) hours, every thirty (30) minutes to seven (7) hours, every hour to fourteen (14) hours, every two (2) hours to twenty-four (24) hours. Readings were taken at twenty-eight (28) hours, thirty-six (36) hours, and forty-eight (48) hours, with subsequent readings every twenty-four (24) hours until the lesions had subsided and healed.

(See Plates 1 - 6, 13 - 14)

### 4-4. Experiment #2 - Equal Weight.

Three (3) men had the gas in liquid form applied to the skin of their left forearms by the platinum pointed drod. The men were cautioned and watched until the gas had visibly disappeared not to move the forearms from a horizontal position. Therefore, any dispersion of gases on the skin was due to the character of the liquid and/or skin; i.e., capillary action, specific gravity, etc. Here it was particularly noted that:

(1) Gases applied on the skin area closest to the wrist immediately began to disperse in the skin folds, causing a linear type of burn. This was particularly true of nitrogen mustard, less so of Lewisite and mustard.

(2) Liquid gas on the other areas on the forearm remained as small circular droplets until complete evaporation took place except for a few small pseudopods which occasionally radiated out from these droplets.

In the application of the liquid gases, as much speed as possible was used so that the three (3) gases were applied almost simultaneously to the forearm. The average overall time of application was three (3) minutes. Any immediate visible reactions were looked for and any

## METHOD OF TEST (Continued)

subjective symptoms noted. The same procedure for pictures and observations were followed as for Experiment 1.

(See Plates 7 - 9, 15 - 16)

### 4-5. Experiment 3 - Equal Volume:

The procedure for this experiment was exactly the same as for Experiment 2 except that the gases were applied by equal volume (0.5 microliters) instead of by equal weight. Again speed of application was stressed so that all three (3) gases were placed on the arm with a minimum of time difference -- average time of application was three (3) minutes.

(See Plates 10 - 12, 17 - 18)

### DATA RECORDED DURING TEST: (Plates 1 - 12)

5-1. The progress of the lesions may best be shown by the use of two (2) graphs for each subject. The first graph shows the lesion developing to a peak, with an insert for the first four (4) hours; the second graph covers the complete reaction to the healing of the lesion.

5-2. In the graphs, no attempt was made to assign to the readings numerical values which would correspond directly to the severity of the lesion. Instead, the ascending and descending readings were merely placed on the abscissa of the graph in the order of their appearance.

5-3. The system used for reading the lesions approaching the peak was the routine one used at the Naval Research Laboratory with the insertion of four (4) additional readings which it was found advisable to use in this experiment.

- (1) E -- Midway between an E- and an E.
- (2) E<sub>+</sub>BP -- An area of E<sub>+</sub> with a blanched papule in the center.
- (3) NV -- Numerous small vesicles forming from an NPV which have not yet coalesced to form one (1) large vesicle.
- (4) V Peak -- The vesicle at its maximum size.

DATA RECORDED DURING TEST (Continued)

5-4. The readings for the descending curve are the most common ones noted in the subsiding and healing lesions. They are self-explanatory.

5-5. For reading the graphs of the complete reaction, use the abscissa readings on the left for the ascending curve, the abscissa readings on the right for the descending curve.

5-6 An attempt was made to show the secondary reactions of Lewisite by a branching of the descending curve. The readings for the three (3) points on this branching are: papules periphery, vesicles periphery, and papules disappeared.

5-7. On all graphs, the solid line represents the mustard lesion, the dotted line the Lewisite lesion, and the broken line the nitrogen mustard lesion.

DISCUSSION

6. In this experiment, twelve (12) men were used; lesions were produced as previously described for each experiment. It was necessary to retain these men for five (5) weeks so that the lesions could be followed through to their conclusion. Certain definite characteristics were noted for each gas:

(1) Immediate reaction:

The first lesion to develop visibly was Lewisite, then nitrogen mustard, and finally mustard. The immediate erythema caused by Lewisite was much more pronounced than that caused by the other two (2) gases. It appeared rapidly and spread immediately over a wide area. It then rapidly diminished and assumed a normal size in comparison to the other two (2) lesions. This is a common characteristic of Lewisite burns and seems to be an easy way to identify this lesion. This may be classed as the immediate or primary reaction, but is no indication of the size of the final lesion as at least one-half ( $\frac{1}{2}$ ) of the diameter disappears leaving no further sign of a burn at that site.

(2) Blanching:

Very close observation was maintained to determine if blanching appeared in the early states of Lewisite contamination. This phenomenon has been observed by other investigators and is said to consist of a blanching of the skin, appearing five (5) to fifteen (15)

## DISCUSSION (Continued)

minutes after Lewisite contamination. We were unable to collaborate these findings as can be seen in the colored photographs and movies. However, these blanching effects did occur later, not only in Lewisite burns but also in mustard burns.

### (3) Vesicles:

The Lewisite vesicle was a tense lesion, and from the medical viewpoint could usually be classified as a so-called pedunculated type; i.e., the diameter of the base was as small as, or smaller, than the diameter of the mid-section. Also, the area of erythema surrounding the base of the vesicle was quite small. The mustard and nitrogen mustard vesicles could be classified as a sessile type of vesicle; i.e., the diameter of the base was larger than any other diameter. These vesicles also, in most cases, did not show the tenseness of the Lewisite lesions, and were surrounded by large haloes of erythema. The larger Lewisite vesicles in some cases appeared cloudy and opalescent. In these vesicles, after a few days, when they had begun to subside, the serum could be palpated as a clotted jelly-like mass.

No evidence of infection was found in the vesicles at any time as proved by bacteriological examination of the serum in the few cases that visibly appeared to be infected. The smears of the serum showed numerous polymorphonuclear leucocytes but no bacteria.

No attempt was made to drain the vesicles, but on the contrary, every attempt was made to preserve them and allow them to subside normally. In a few cases, they ruptured spontaneously, but most were preserved. In all except one (1) man, every burn resulted in the formation of a vesicle. This man was apparently highly resistant and could not be considered as a typical case.

### (4) Severity of Lesions:

In the overall picture, the mustard lesions were the most pronounced. This was true not only for the diameter, but also for the depth of the lesion. Mustard lesions definitely injured the skin and caused, in most cases, third degree burns, exposing, and in some cases, damaging the corium. Lewisite lesions are next in severity

## DISCUSSION (Continued)

severity, causing in many cases third degree burns, but usually the corium was spared. The nitrogen mustard lesions were comparatively superficial and resulted mostly in second degree burns, injuring the superficial and intermediate skin layers.

### (5) Secondary Reactions:

The only secondary reactions observed were caused by Lewisite and occurred approximately fourteen (14) days after the original burn. These were evidenced by marked erythema around the original burn area, and the appearance of numerous small papules and vesicles surrounding the primary burn site. These small vesicles in the majority of the cases formed a halo around the original lesion. They were accompanied by intense itching but no pain.

### (6) Pain:

Lewisite caused pain almost immediately after application. The mustard and nitrogen mustard lesions were practically painless. Any later discomforts were due to the lesions themselves and not to the causative agent.

### (7) Position:

Those lesions at the center position on the forearm tended to peak more rapidly than those at the other two (2) positions. However, the final size and severity of the lesions were not significantly affected by the position of the lesion on the arm except that the lesions at the wrist were invariably of a linear type.

## RESULTS

7. (1) Immediate itching and burning appeared with Lewisite, occasional itching with nitrogen-mustard, and no immediate symptoms with mustard.

(2) The mustard and Lewisite vesicles were approximately the same in size. The nitrogen mustard vesicles were generally smaller.

(3) The average lengths of time required by the lesions to reach a peak (maximum vesication) were thirty-nine (39) hours for mustard and forty-two (42) hours for Lewisite and nitrogen mustard.

## RESULTS (Continued)

(4) In comparing lesions by weight with lesion by volume, the nitrogen mustard by weight were larger than the nitrogen mustard by volume. No significant difference was observed between the mustard weight and volume lesions or the Lewisite weight and volume lesions.

(5) All three (3) agents produced a linear type of lesion when applied to the wrist areas.

(6) Those lesions whose roofs remained intact, although they may previously have been ruptured, healed more quickly than those lesions which became denuded, and formed a true scab later.

(7) The time of healing of the lesions was as follows: nitrogen mustard first, Lewisite second, and mustard last, irrespective of the secondary reactions from Lewisite.

(8) The nitrogen mustard lesions showed the most induration (edema).

(9) Pigmentation of the skin appeared first in nitrogen mustard, then mustard, and last Lewisite. Pigmentation was most pronounced in nitrogen mustard, less in mustard, and least in Lewisite.

(10) Varying degrees of concavity were observed in the mustard and nitrogen mustard lesions after the formation of scabs, the mustard lesions being the most pronounced.

(11) The secondary reactions from Lewisite appeared about fourteen (14) days after the onset of the initial lesion. Thirty-one (31) days later these reactions were still evident. No secondary reactions appeared with mustard and nitrogen mustard.

(12) The final scars were most severe with mustard, less with Lewisite, and least with nitrogen mustard.

(13) No constitutional symptoms could be demonstrated at any time by routine physical examinations and laboratory procedures.

## CONCLUSIONS

8. (1) Mustard gas causes the most severe lesions, Lewisite is next in severity, and nitrogen mustard the least. The healing time is in the reverse order.

(2) The average peak lesion is reached in thirty-nine (39) hours with mustard, and in forty-two (42) hours with Lewisite and nitrogen mustard.

(3) The Lewisite vesicle can be distinguished from the mustard and nitrogen mustard vesicles by its pedunculated appearance and the tendency of the serum to coagulate within the vesicle.

(4) The nitrogen mustard lesions produced by weight (1 milligram of the agent) are larger than those produced by volume (0.5 microliters of the agent).

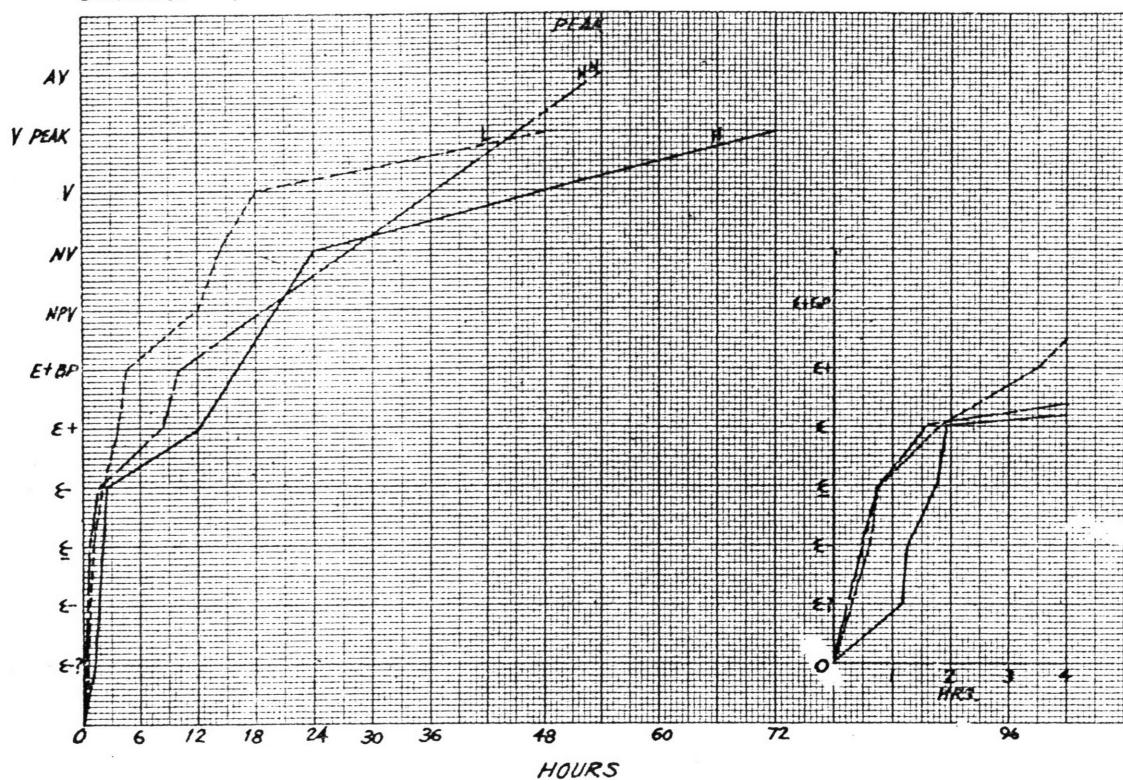
(5) Pigmentation of the skin appears first and is most pronounced in the nitrogen mustard lesions.

(6) Lewisite causes an immediate erythema over a wide area which rapidly diminishes and assumes a normal size in comparison to the other lesions.

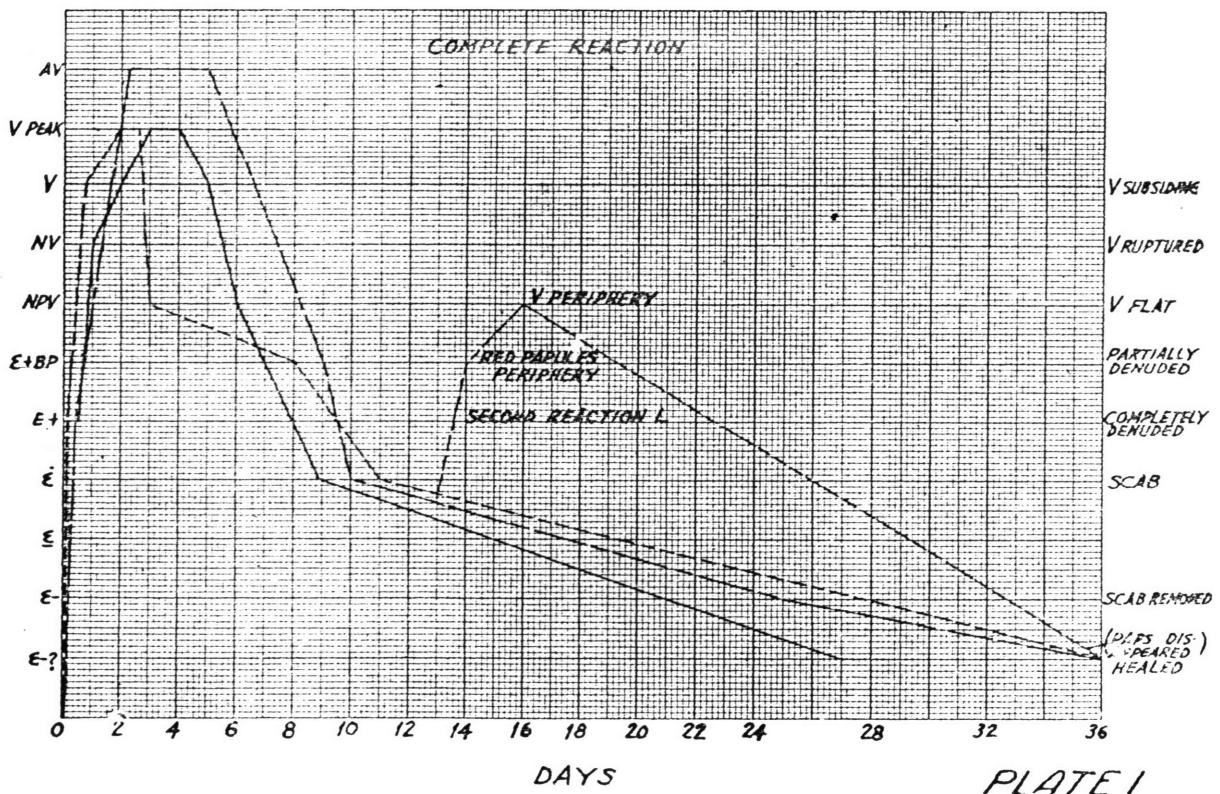
(7) Those lesions whose roofs remain intact heal more quickly than those which become denuded and form a true scab later.

(8) The center position on the forearm is slightly more sensitive than the other two (2) positions. Those lesions at the wrist position are linear in character.

SUBJECT #1



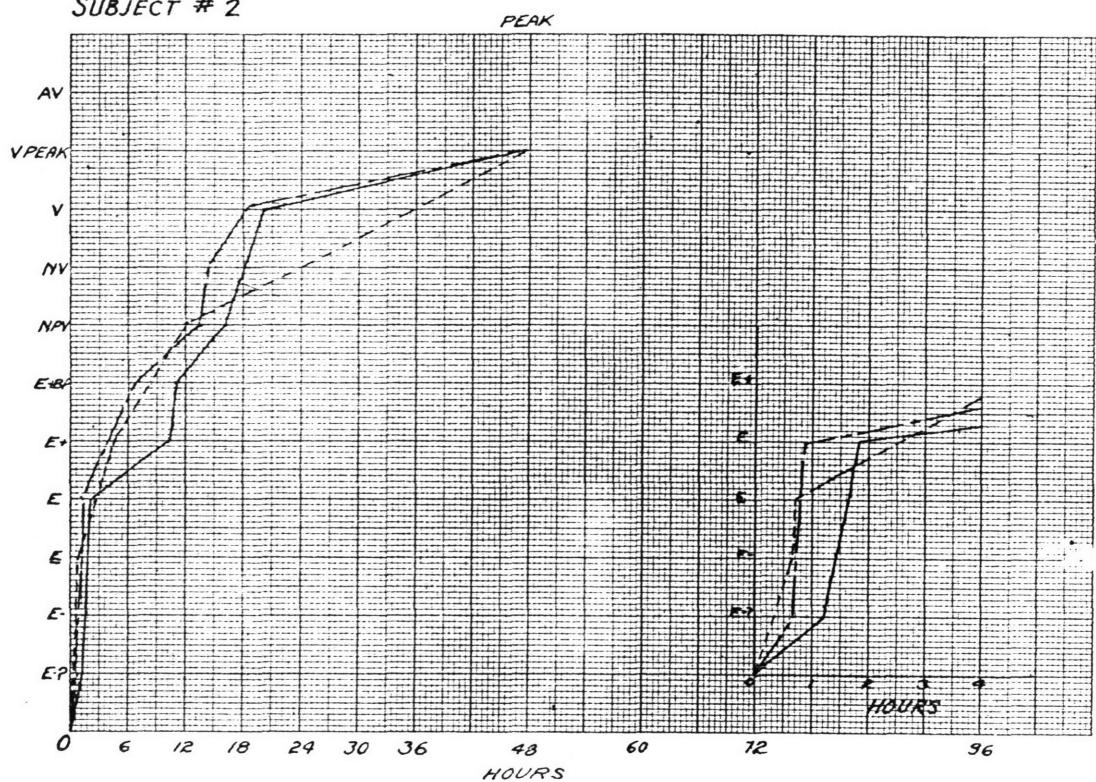
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SUBJECT #1



DAYS

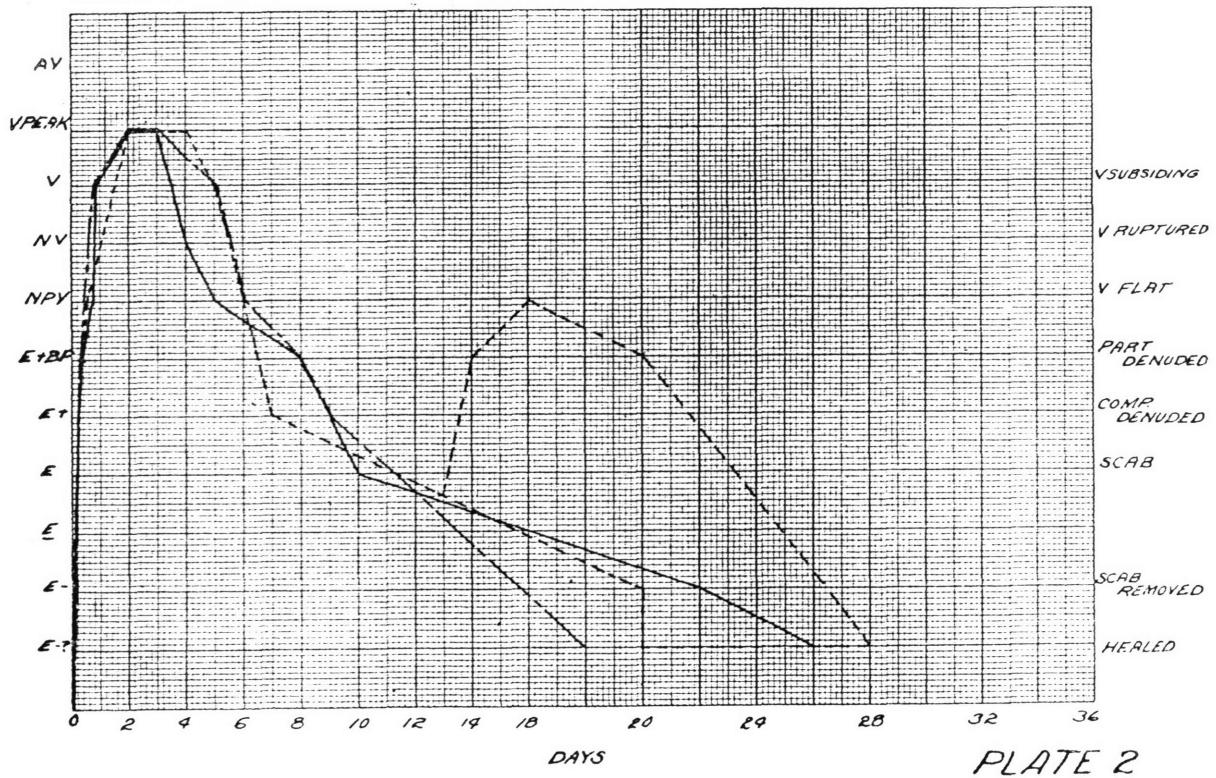
PLATE 1

EXP. # 1  
SUBJECT # 2



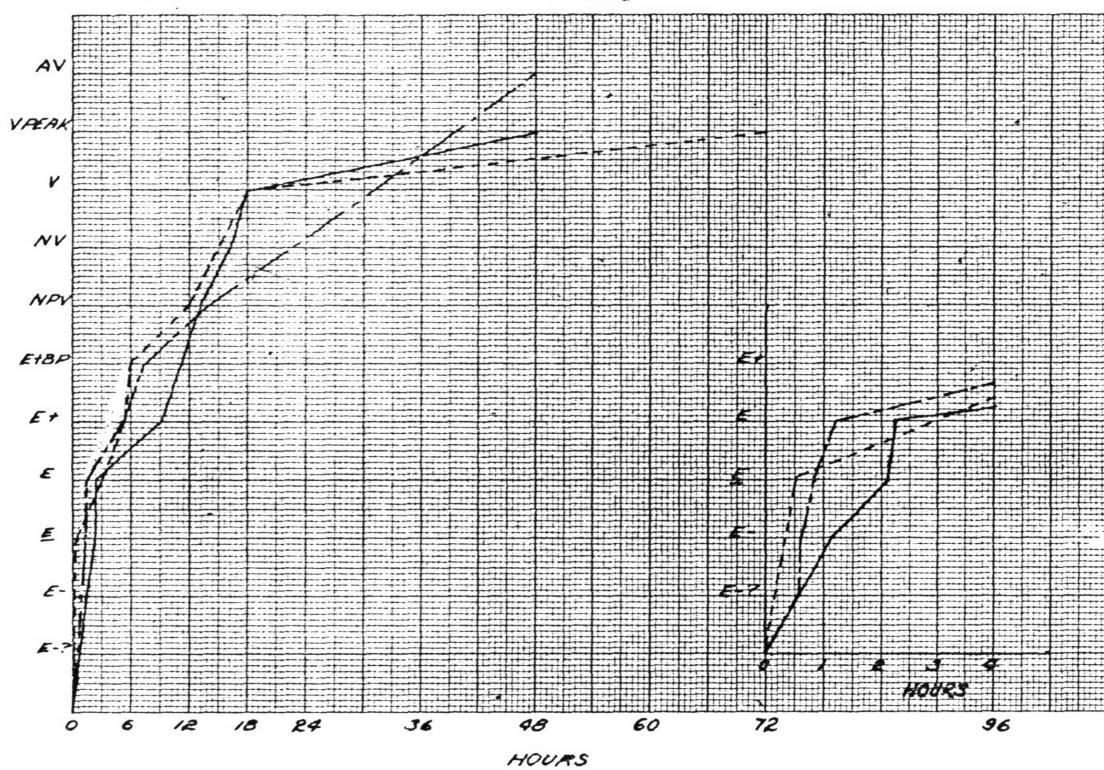
EXP. # 1  
SUBJECT # 2

COMPLETE REACTION



EXP. # 1  
SUBJECT # 3

PEAK



EXP. # 1  
SUBJECT # 3

COMPLETE REACTION

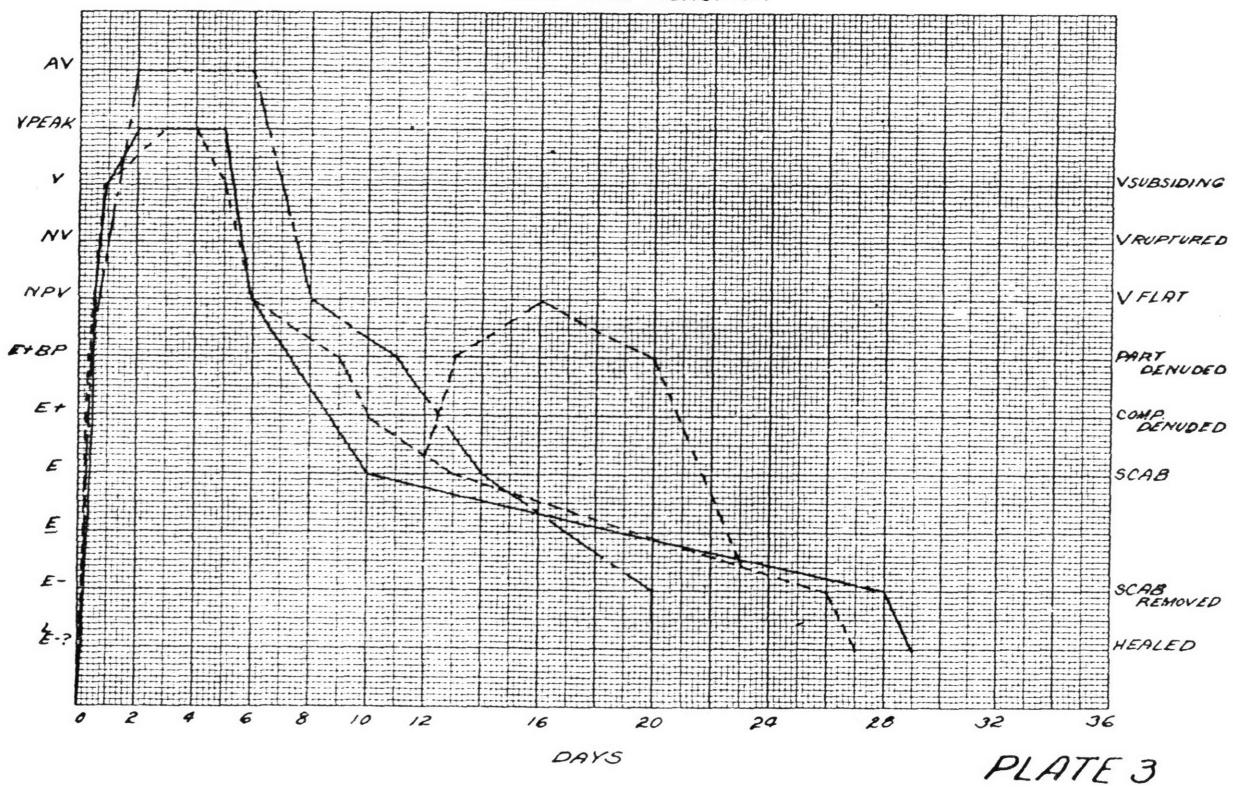
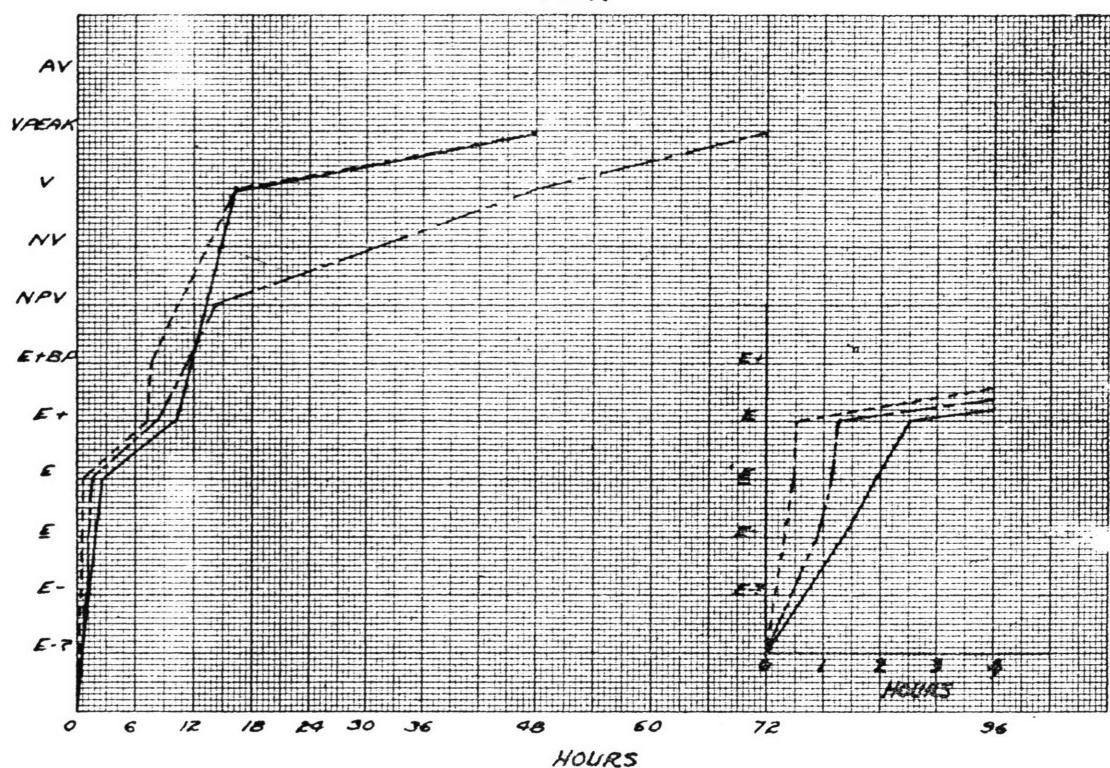


PLATE 3

EXP. # 1  
SUBJECT # 4

PEAK



EXP. # 1  
SUBJECT # 4

COMPLETE REACTION

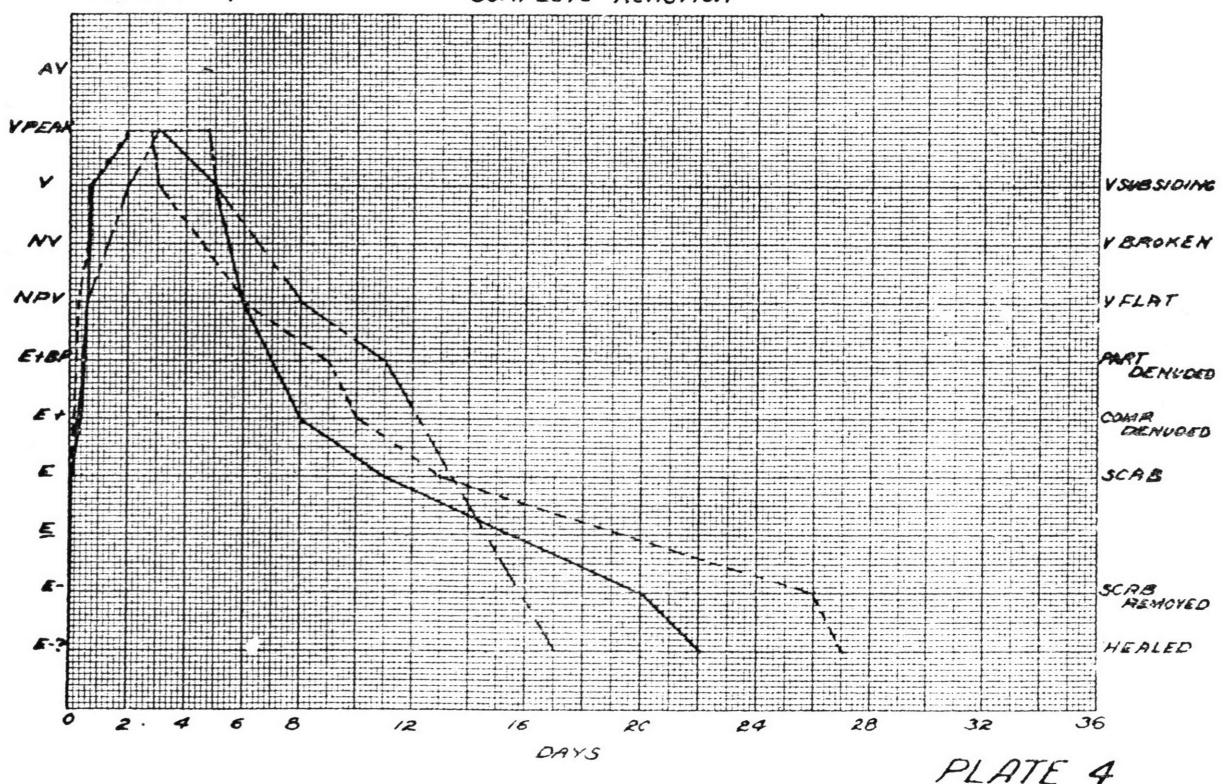
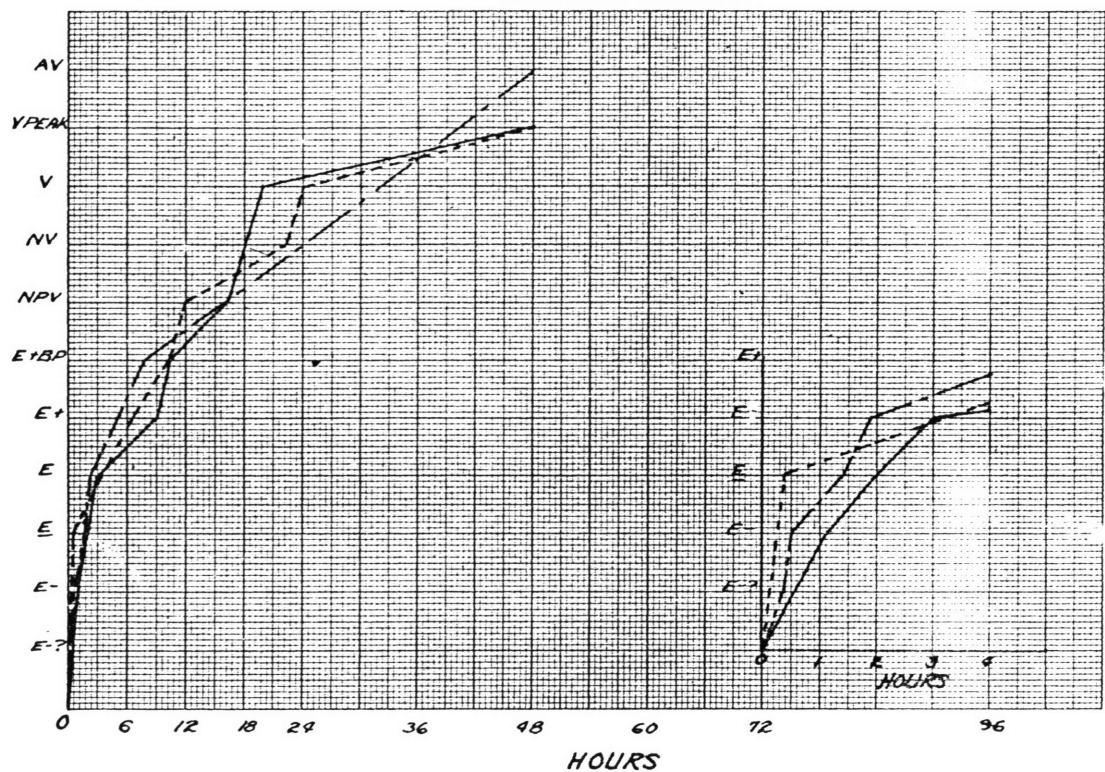


PLATE 4

EXP. # 1  
SUBJECT #5

COMPLETE REACTION



EXP. # 1  
SUBJECT #5

COMPLETE REACTION

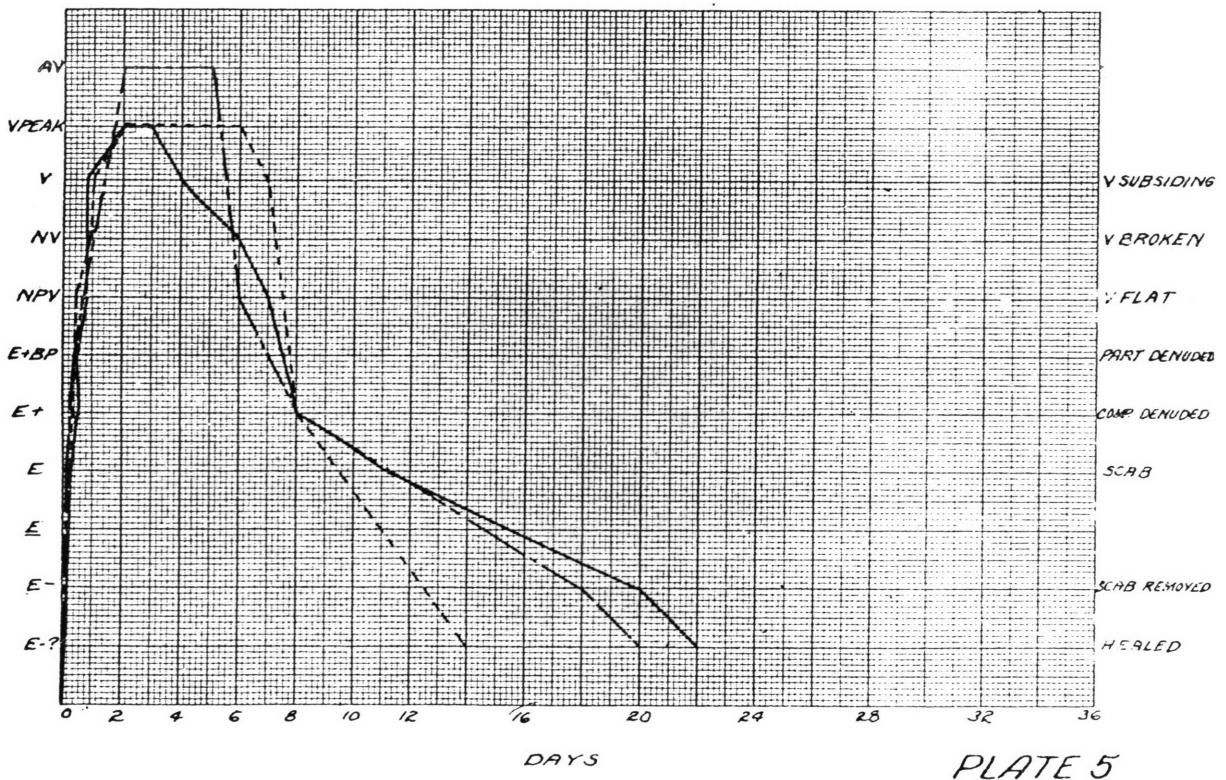
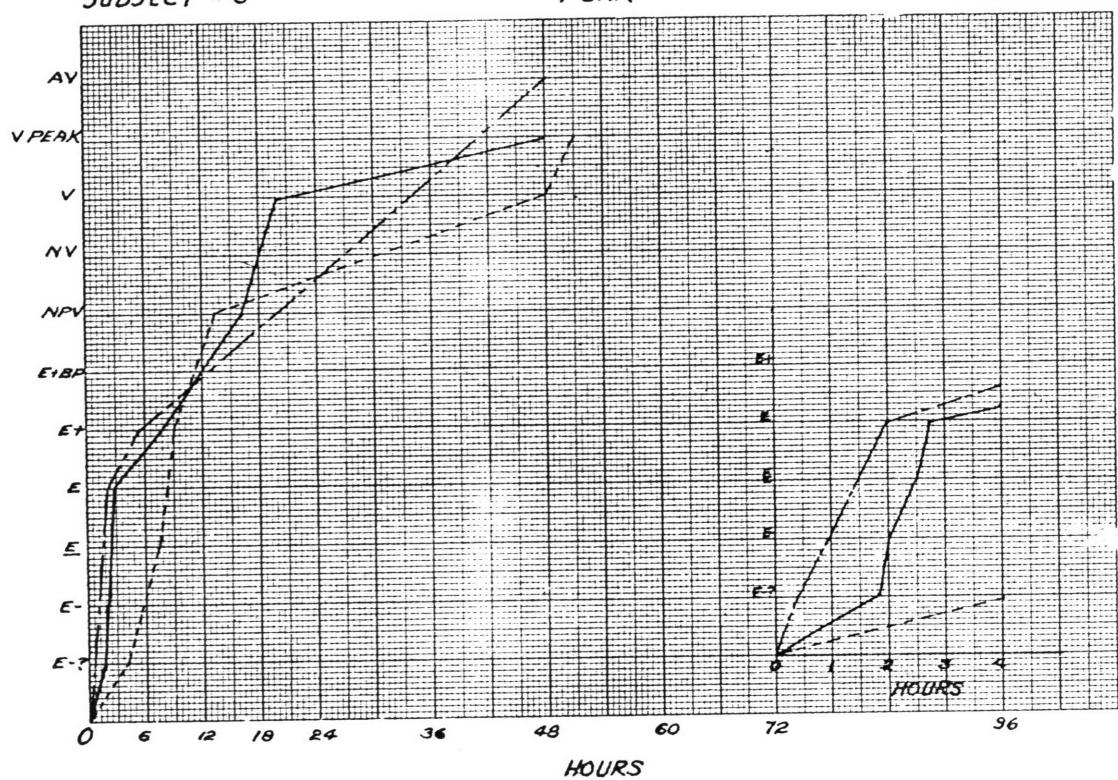


PLATE 5

EXP. #1  
SUBJECT #6

PEAK



EXP. #1  
SUBJECT #6

COMPLETE REACTION

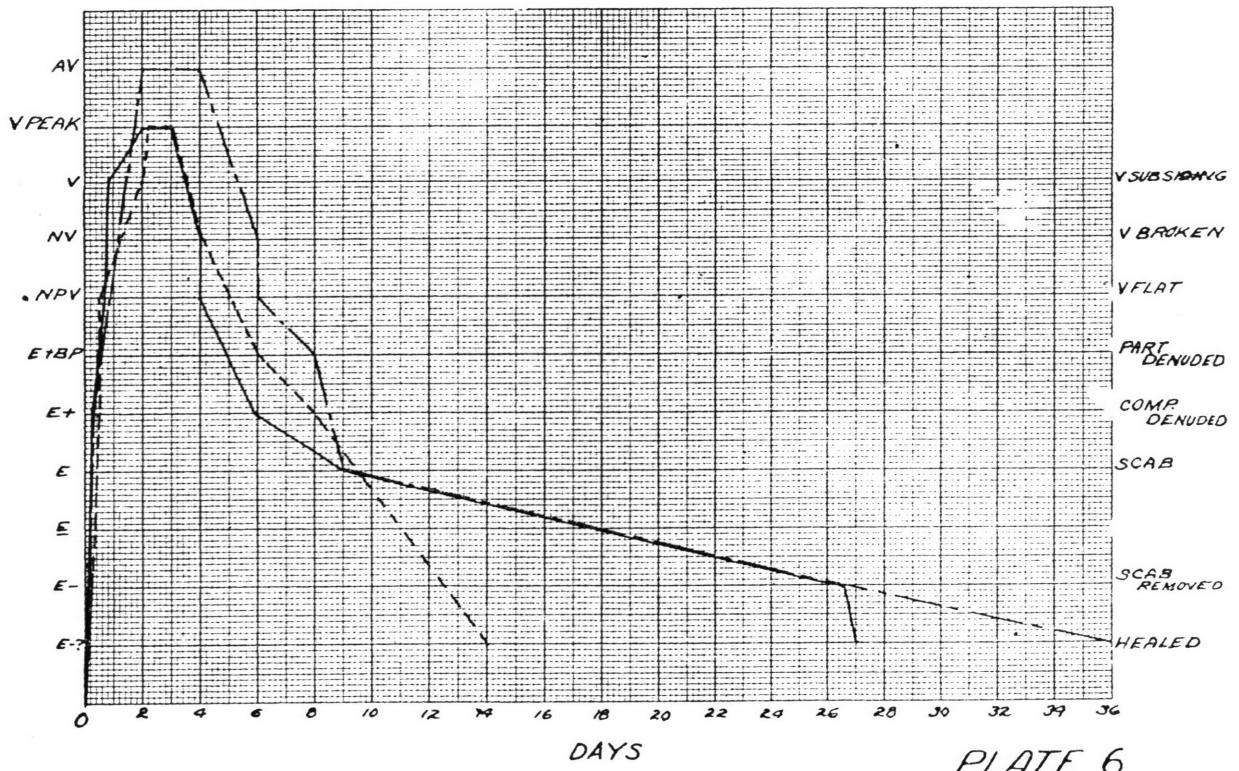
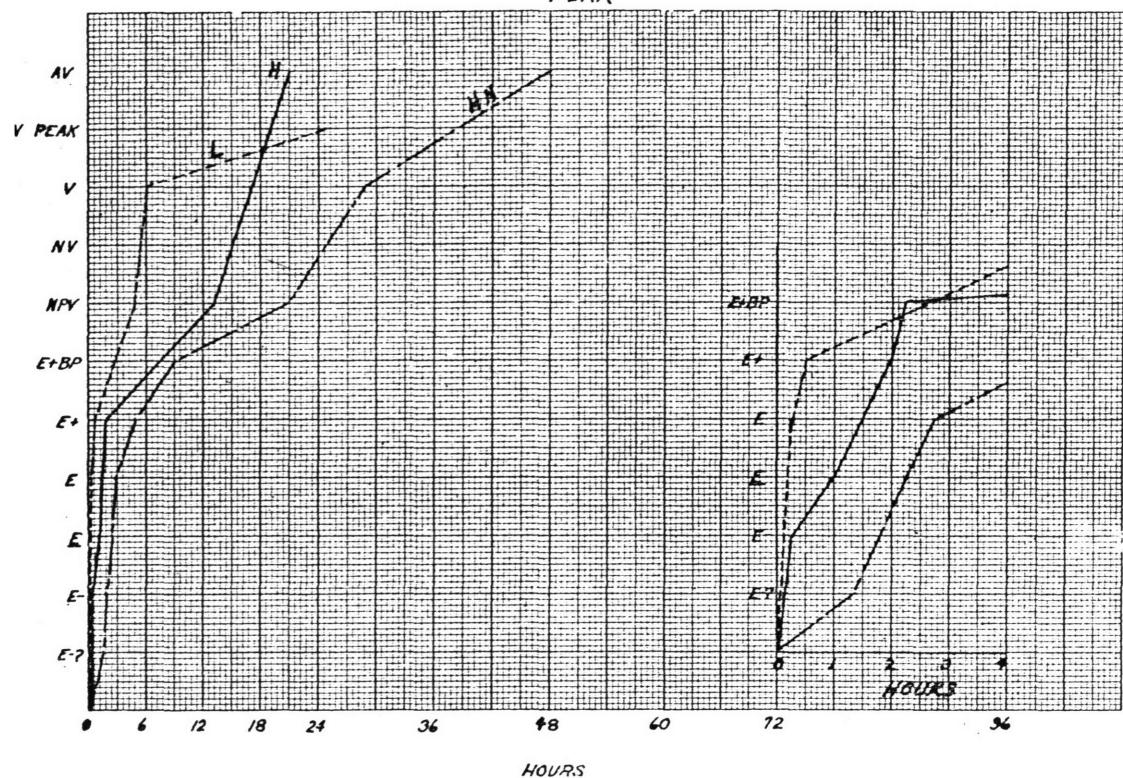


PLATE 6

EXP. # 2  
SUBJECT #7

PEAK



EXP. # 2  
SUBJECT #7

COMPLETE REACTION

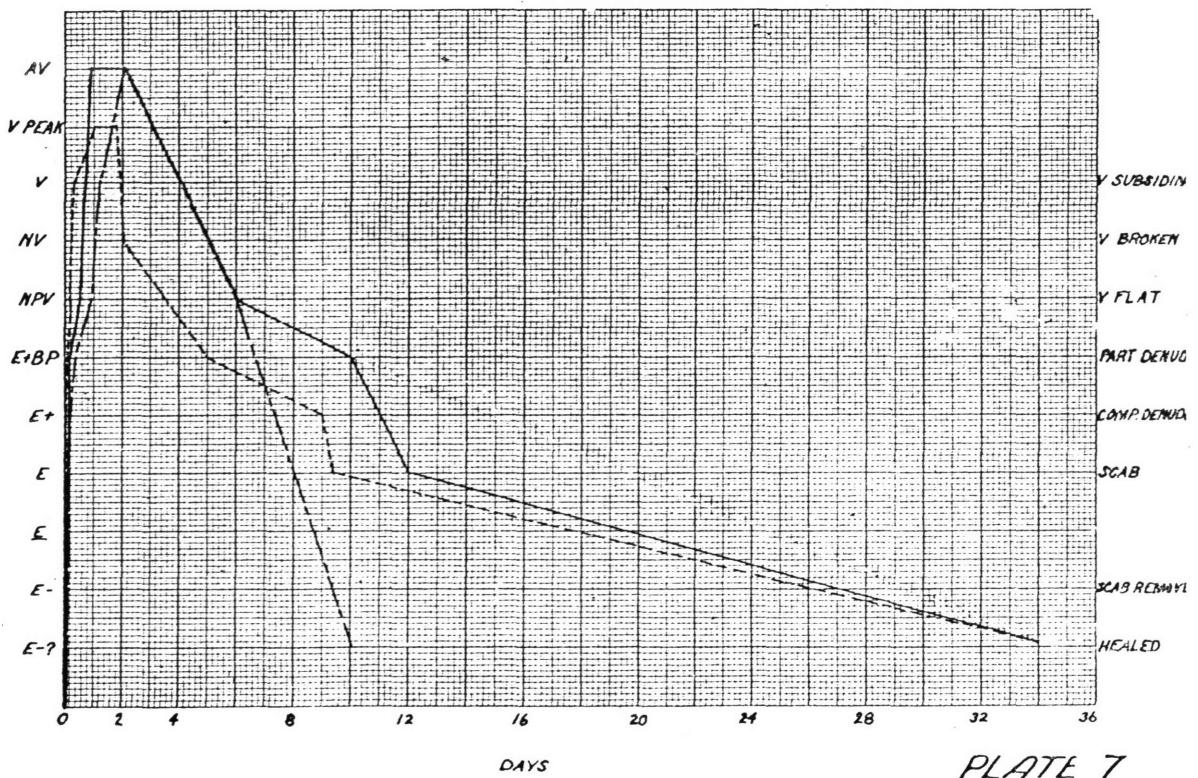
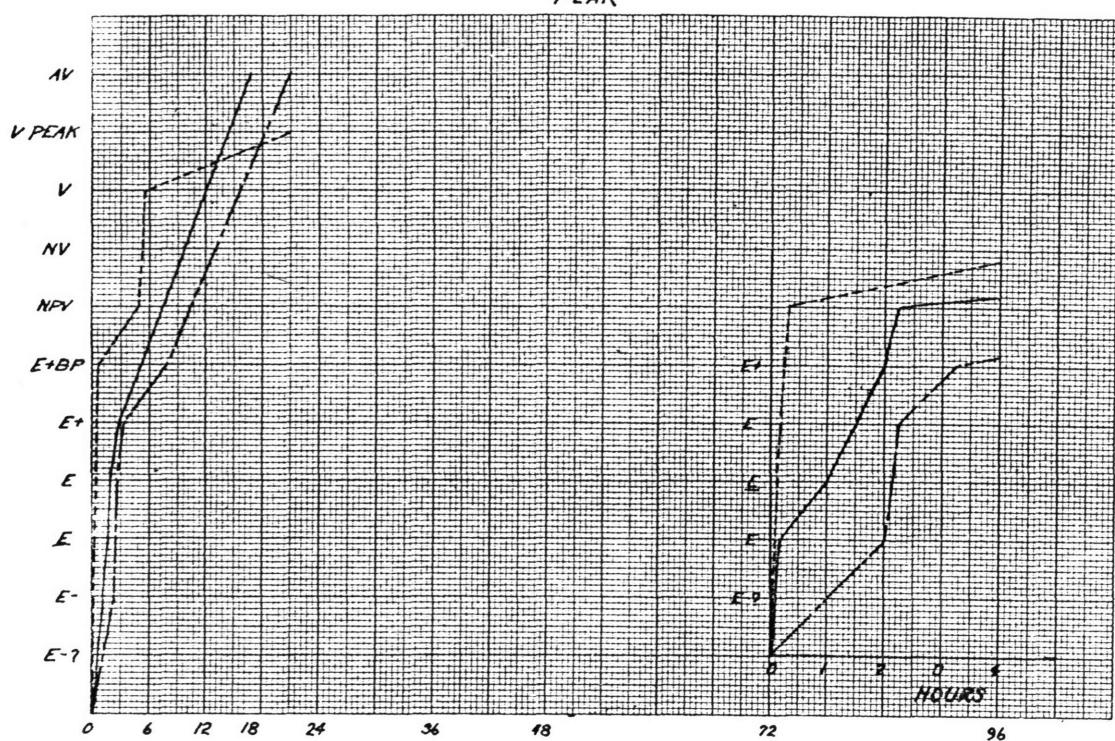


PLATE 7

EXP. # 2  
SUBJECT # 8

PEAK



EXP. # 2  
SUBJECT # 8

COMPLETE REACTION

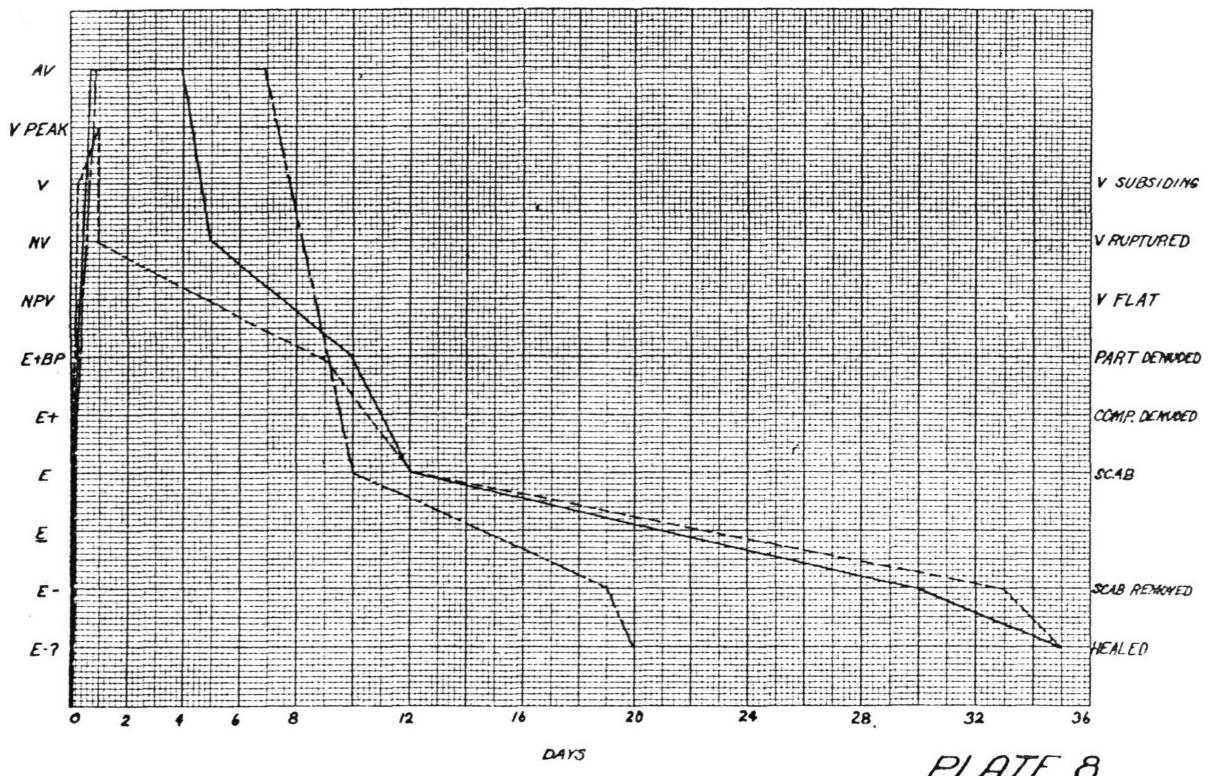
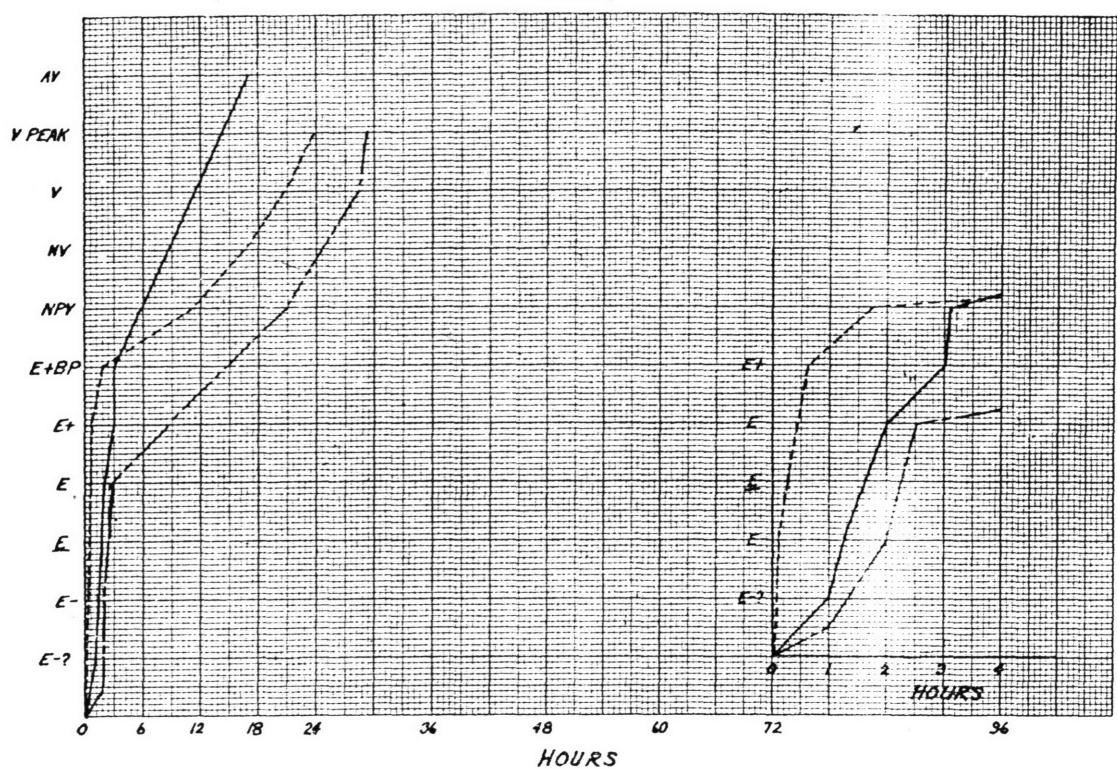


PLATE 8

EXP. # 2  
SUBJECT # 9

PEAK



EXP. # 2  
SUBJECT # 9

COMPLETE REACTION

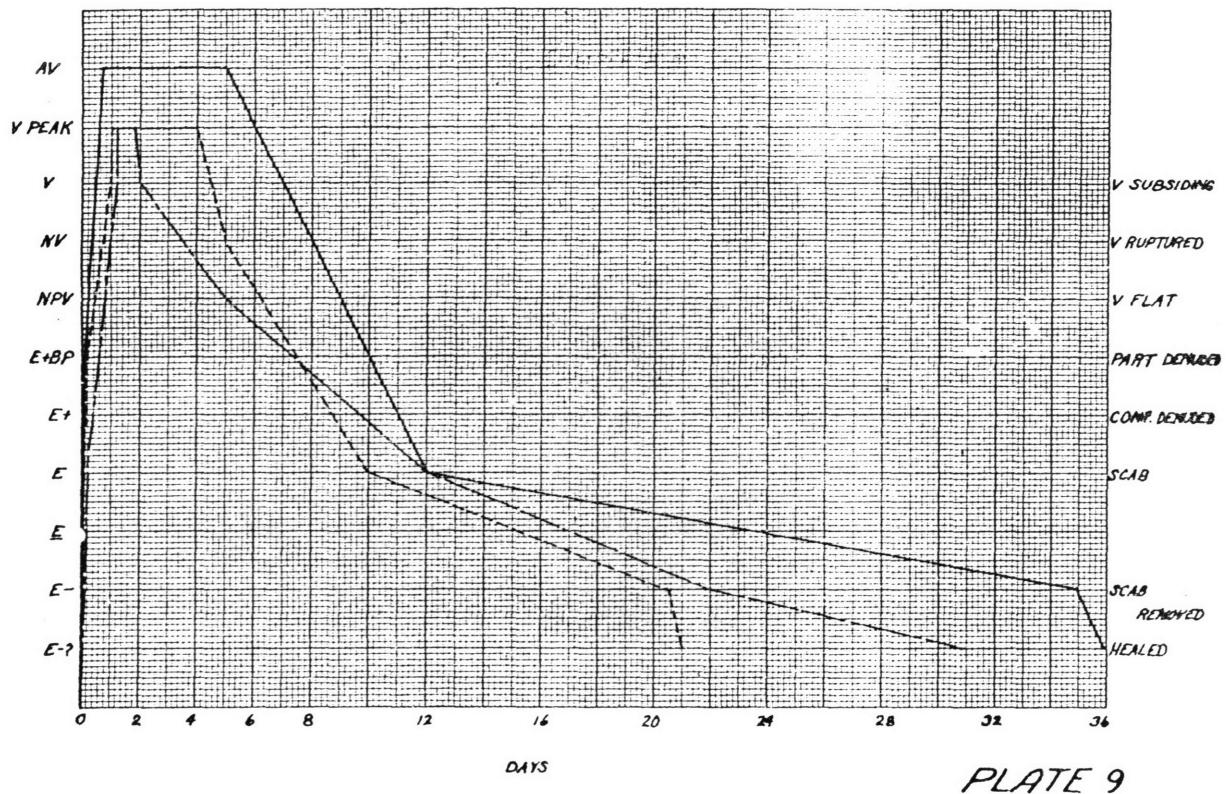
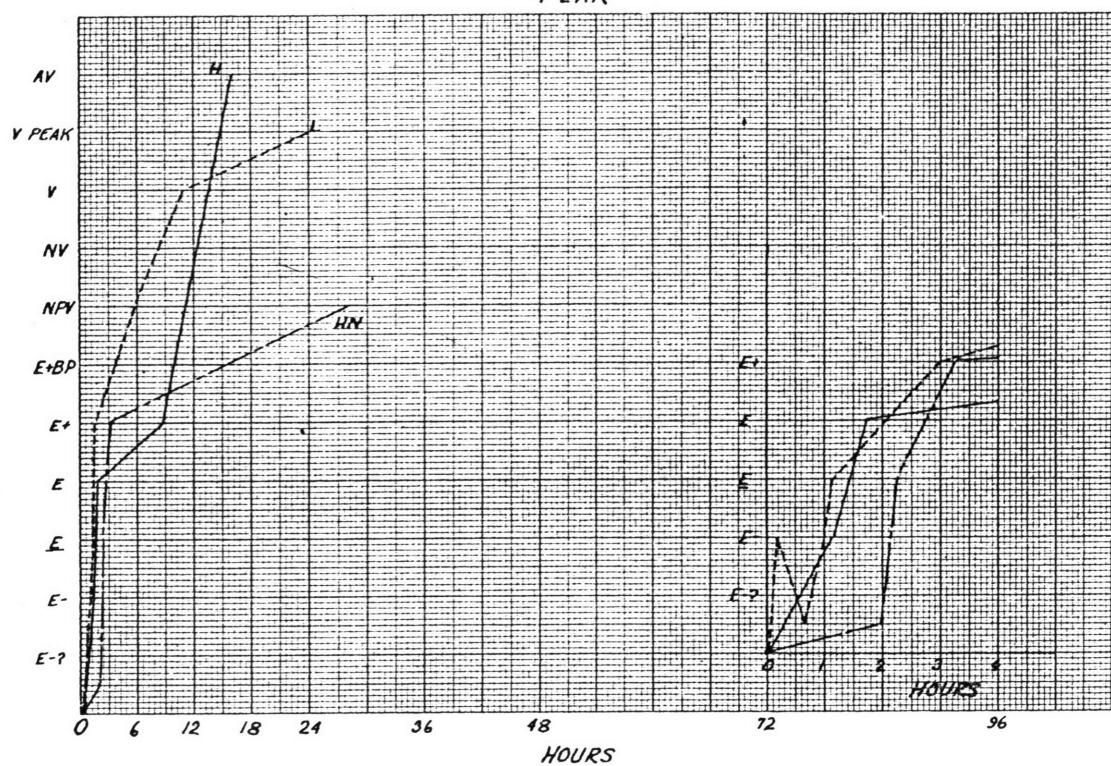


PLATE 9

EXP. # 3  
SUBJECT # 10

PEAK



EXP. # 3  
SUBJECT # 10

COMPLETE REACTION

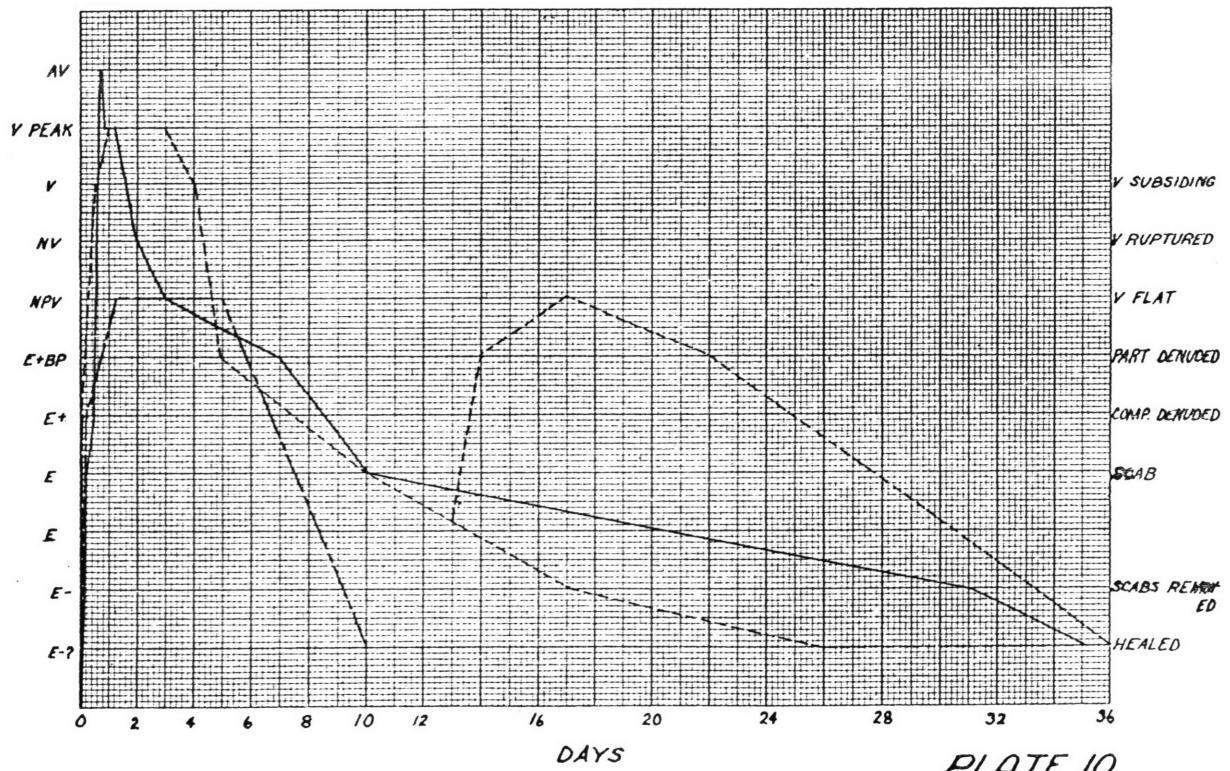
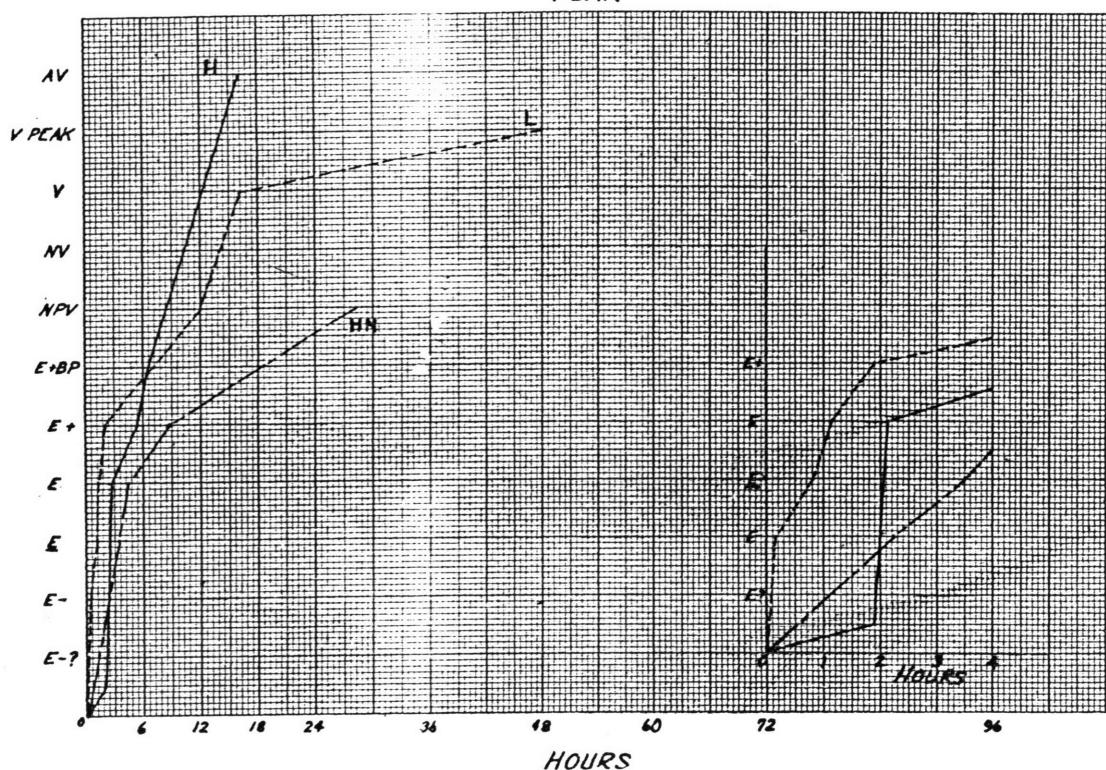


PLATE 10

EXP. # 3  
SUBJECT # 11

PEAK



EXP. # 3  
SUBJECT # 11

COMPLETE REACTION

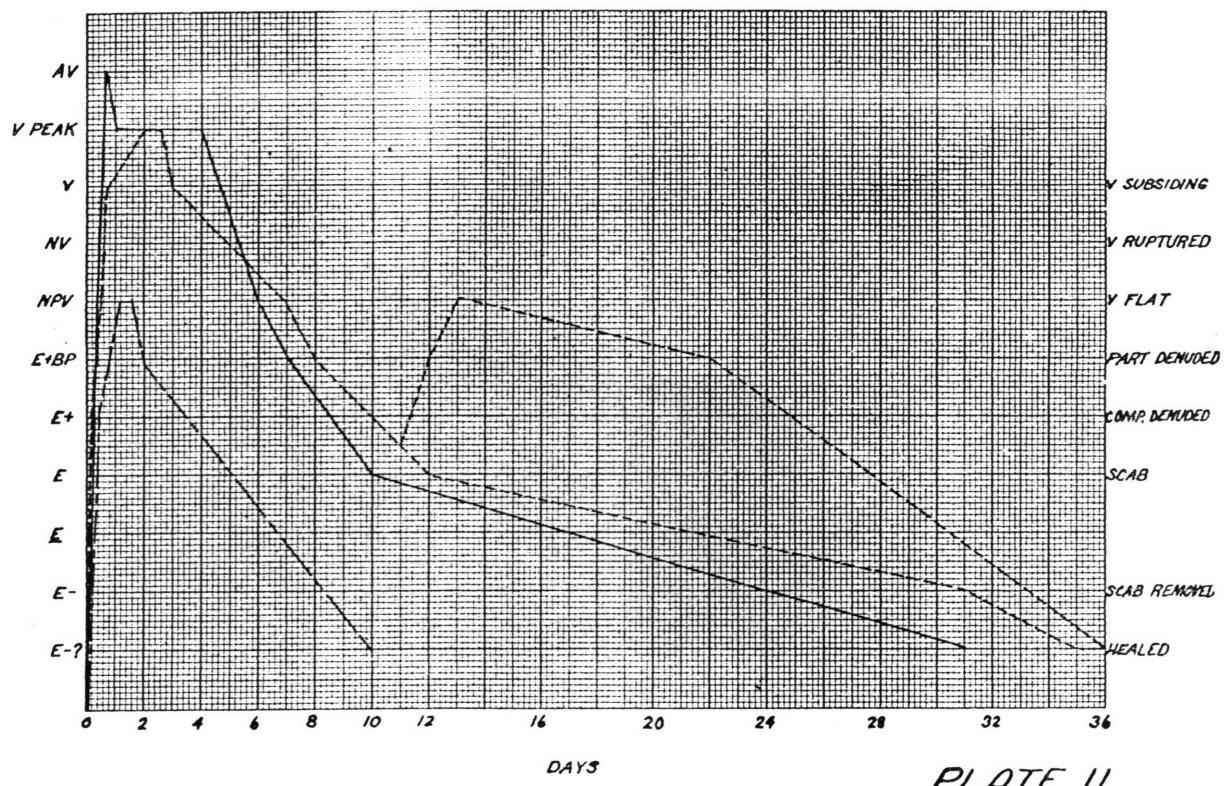
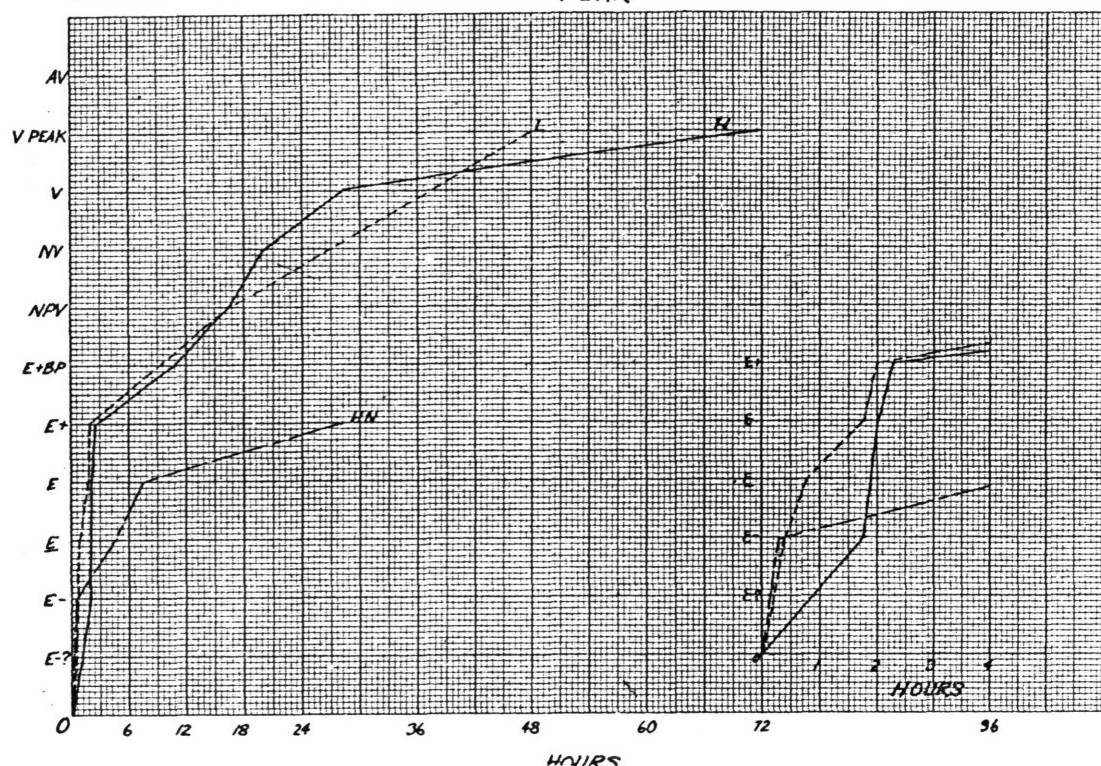


PLATE 11

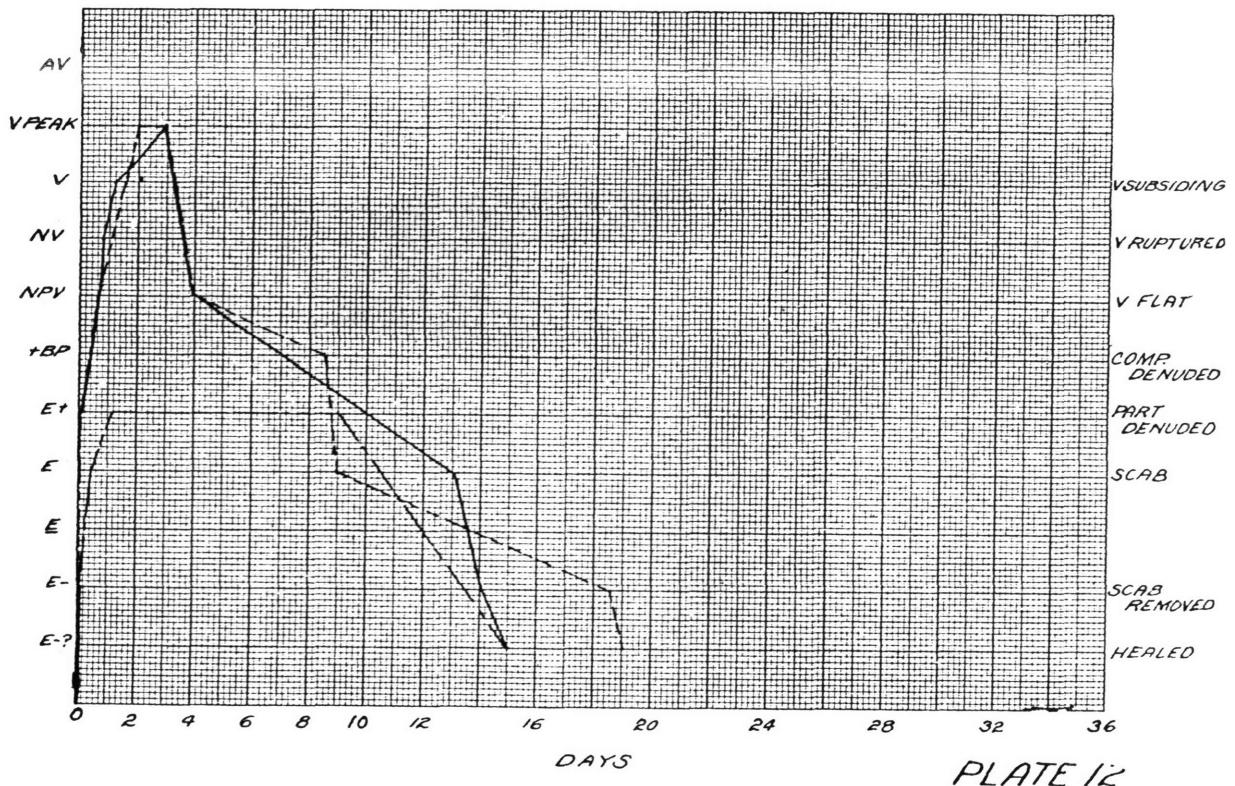
EXP. # 3  
SUBJECT #12

PEAK

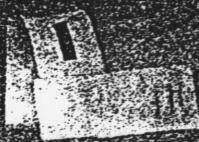


EXP. # 3  
SUBJECT # 12

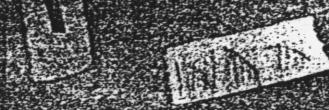
COMPLETE REACTION



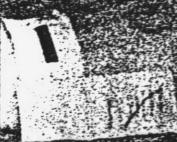
EXPERIMENT NO. 1 REPRESENTATIVE MAN USING VAPOR CUPS. MAN NO. 1  
H (PROXIMAL) L (MIDDLE) HN (DISTAL)



1 1/2 Hours



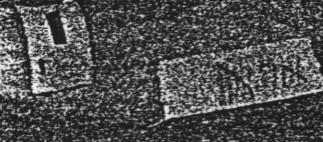
24 Hours



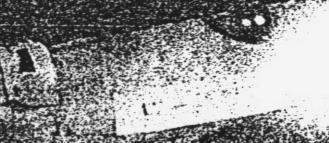
4 Hours



36 Hours



8 Hours



48 Hours



20 Hours



54 Hours

Plate 13

72 HR

72 Hours

13 DAYS

13 Days

120 HR.

120 Hours

16 DAYS

16 Days

144 HR

144 Hours

21 DAYS

21 Days

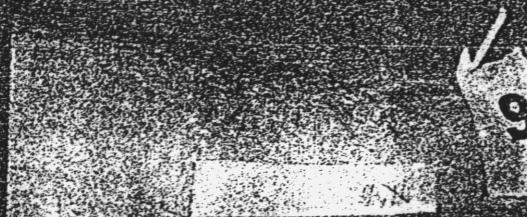
8 DAYS

8 Days

27 DAYS

27 Days

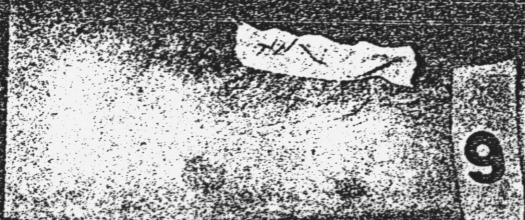
REPRESENTATIVE MAN IN EXPERIMENT NO. 2 USING WEIGHT. MAN NO. 9  
HN (PROXIMAL) H (MIDDLE) L (DISTAL)



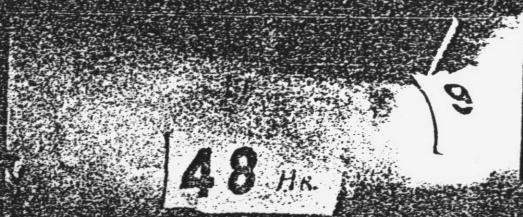
2 1/2 Hours



28 Hours



8 Hours



48 Hours



10 Hours



72 Hours



21 Hours



72 Hours

Plate 15

96

96 Hours

12

12 Days

120

120 Hours

15

15 Days

7 DAYS

7 Days

20

20 Days

9

8 DAYS

8

8 Days

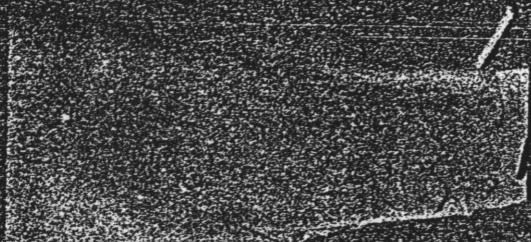
22 DAYS

9

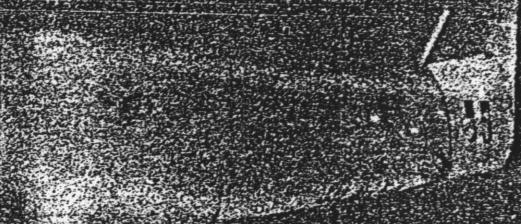
22 Days

Plate 16

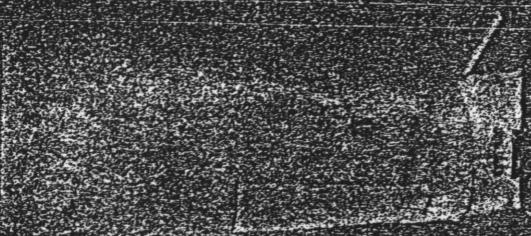
REPRESENTATIVE MAN IN EXPERIMENT NO. 3 USING VOLUME. MAN NO. 11  
L (PROXIMAL) M (MIDDLE) R (DISTAL)



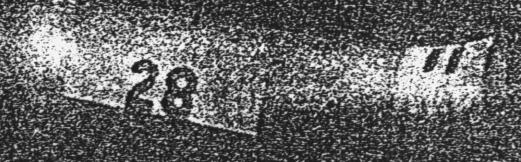
2 Hours



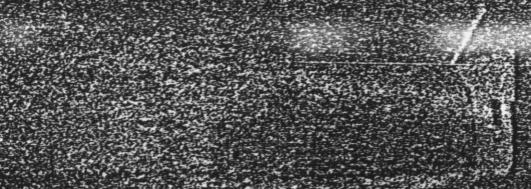
20 Hours



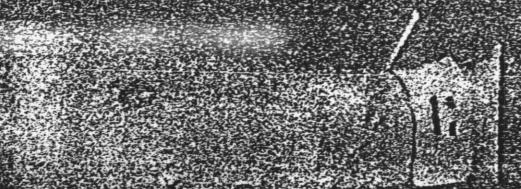
6 Hours



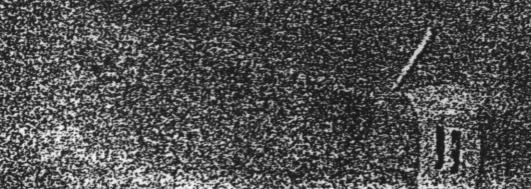
28 Hours



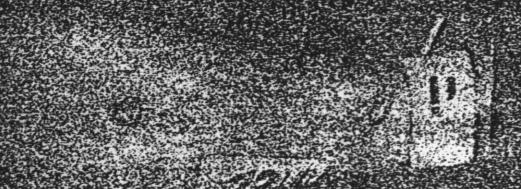
9 Hours



48 Hours



16 Hours



72 Hours

Plate 17

96 //

96 Hours

10 DAYS //

10 Days

120 //

120 Hours

15 DAYS //

15 Days

144 //

144 Hours

20 DAYS //

20 Days

7 DAYS //

7 Days

23 DAYS //

23 Days